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SUSTAINED PROTECTION AGAINST SUPERFICIAL BACTERIAL AND FUNGAL INFECTION BY TOPICAL TREATMENT

FINAL COMPREHENSIVE REPORT

by

Albert M. Kligman, M.D., Ph.D.

31 December 1974

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Washington, D.C. 20314

Contract No. DADA 17-71-C-1009

University of Pennsylvania
Philadelphia, Pennsylvania 19104

DDC AVAILABILITY STATEMENT

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The findings in this report are not be construed as an official Department of the Army position unless so designated by other authorized documents.

ABSTRACT

This report summarizes investigations

(1) delineating simple reproducible bioassays for measuring bacteriostatic and bacteriocidal activity of topical antimicrobial agents;

(2) delineating pitfalls in using the paired-comparison type of analysis for topical antibacterials;

(3) quantifying the bacteriology of two common dermatoses, atopic dermatitis and psoriasis, and the effect of antibiotic therapies in these disorders;

(4) delineating experimental models for the evaluation of steroid-antibiotic combinations;

(5) describing a technique for experimental *Candida albicans* infections and its usefulness in evaluating anti-candida therapies;

(6) describing the microbiology of the various species of interdigital eruptions of the feet and studies on various therapies for this condition; and

(7) delineating *in vitro* and *in vivo* tests for the antimicrobial efficacy and stringent effects of various aluminum salts.

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Hundreds of topical products are offered which claim to be able to eradicate cutaneous micro-organisms. These fall into various use categories: 1) prevention and treatment of infection; 2) preparative scrubs for minor and major surgery; 3) first-aid creams and wound cleansers; 4) anti-bacterial soaps; 5) surgeon's scrub.

Many of these are now receiving critical review by the F.D.A. because supporting data is inadequate and in many cases lacking altogether. There is no question that the active ingredients possess bacteriostatic activity. The difficulty is that the data chiefly relate to in vitro tests. The in vivo circumstances are quite different and indeed very variable. Exudates inactivate some bacteriostats. Skin secretions, especially sebum, may complex with many compounds. Hexachlorophene, for example, is much less effective on the scalp than elsewhere owing to surface lipids. The jump between the test tube and the skin surface is large.

Many time-honored potent bacteriostatics are of doubtful efficacy on the skin itself; these include, quaternary ammonium compounds (widely used by laymen to treat minor cuts, wounds and abrasions), heavy metals (mercury), phenol, resorcinol, etc. Moreover, when one considers the great variety of circumstances under which these agents are used, it becomes obvious that no single in vivo assay will encompass all contingencies. Granting that a substance has been demonstrated by a particular technique to suppress cutaneous micro-organisms, it must still be ascertained that it satisfies the claims made for it. For example, bacteriostatic soaps decrease the population density of resident organisms in the hands and forearms. However, it cannot be inferred from this demonstration that regular use will prevent pyodermas caused by virulent streptococci and staphylococci. It is a formidable enterprise to establish the prophylactic value in field studies. The results obtained so far in clinical studies of bacteriostatic soaps are highly controversed.

Our approach has been to devise bioassays to obtain the specific kinds of data appropriate to various intended uses. Using a stepwise sequence of tests on small groups of human volunteers, one secures a "profile" of the bacteriostatic which permits a reasonable estimate of how it will perform in the clinical setting.

Objective methods are used which yield quantitative data on the following key concerns:

a. bacteriostatic activity;

b. bacteriocidal activity;

c. residual activity (substantivity);

d. ecologic changes;

e. activity in the presence of serum; and

f. effectiveness in different body regions.

PROCEDURES FOR BIOASSAY

I. Bacteriostatic Activity - The Occlusion Test

This test is based on the fact that occlusion of normal forearm skin with an impermeable plastic film results in an explosive increase in the density of resident micro-organisms from hundreds per square centimeter to hundreds of thousands in a 48-hour period. The increased surface temperature and the accumulation of water provide the stimulus for this growth. An effective antibacterial substance will prevent this expansion. The use of bacteriostatic toiletries and cosmetics is prohibited for at least a week before the test. 5 cm. squares (total area of 25 sq. cm.) are marked out on the forearms of ten volunteers. A 0.1 ml volume of each test agent, or 0.1 ml of creams and ointments delivered through a tuberculin syringe, is distributed over the square. The area is covered with Saran wrap and sealed to the skin with adhesive tape for 48 hours. The quantity of organisms is determined by plating out bacteria obtained by the detergent-scrub method of sampling. A control site must demonstrate a density of at least 10,000 organisms/cm.,^{2} indicating that occlusion was complete. Low numbers signify partial dehydration and could result in falsely attributing bacteriostatic activity to an ineffective compound. The usual level attained is about 10^5 organisms/cm.^{2} The potency of a bacteriostatic substance can be assayed by determining the minimum concentration per sq. cm. of skin that prevents expansion of the microflora. The agent is dissolved in water or a volatule solvent such as ethanol to exclude vehicle effects. Some typical results are given in Table I.

TABLE I

ASSAY OF BACTERIOSTATIC POTENCY

10 Subjects. Mean density/cm.^{2}

- 48 hours' occlusion -

Antibacterial Substance	1% 40mg/cm.^{2}	0.1% 4mg/cm.^{2}	0.01% 0.4mg/cm.^{2}	Control
Bacitracin	173	162	514	188,000
Chloramphenicol	120	595	204	17,000
Hexachlorophene	312	1790	1750	10,400
Benzalkonium chloride	10	73	2880	25,800
Neomycin	322	430	1200	146,000
Povidone iodine	951	20,100	98,600	414,000
Pyrithione Sodium	158	673	6760	109,000

Values enclosed by bars are not significantly different by analysis of variance.

It will be seen that all of these were highly inhibitory of 40 mg/cm.^{2} and with the exception of povidone iodine were still very active at 0.4 mg/cm.^{2} By our standards, the effect is not significant unless a ten-fold reduction is achieved (one order of magnitude). To date, no substance has rivalled the potency of antibiotics such as chloramphenicol and neomycin.

The bacteriostatic efficacy of formulated products can also be evaluated. Table II shows the results for a number of familiar proprietaries and other agents.

TABLE II

ASSAY OF ANTIBACTERIAL FORMULATIONS

Density cm.^{2} - Ten Subjects.

(48 hours' occlusion)

Material	
Control	1,400,000
70% Alcohol	1,700,000
Tincture of iodine	200,000
Basic Fuchsin	9,500
Castellani's Paint	200
Crystal Violet (1%)	100
Merthiolate	85,000
Neosporin cream (neomycin, gramicidin, polymyxin)	78
Polysporin ointment (bacitracin, polymyxin)	450
Vioform cream	1,750
1% Haloprogin cream	220,000
0.1% Gentamycin cream	460
5% Haloprogin cream	2,383

As expected, the antibiotic creams were highly effective. The Triphenol dyes, long used in medicine, were very potent. Ethenol had no effect. This is not surprising for it disappears rapidly. Agents which have immediate killing power but no residual activity have to be assayed in a different way (see below). The test appears to be fairly discriminating; 5% Haloprogin was effective but 1% was not.

This procedure is also valuable for determining antibacterial properties of the vehicle. We find, for example, that some of the newer steroids, formulated in propylene glycol gels, are moderately bacteriostatic.

When only modest activity is demonstrated in the 48-hour assay, it is desirable to reduce the time to 24 hours, to reveal agents whose residual effect may not be so great. Tincture of iodine for example becomes much more effective in the 24-hour test. Iodine complexes with organic substances on the skin surface.

One of our concerns is to find bacteriostatic and fungi static substances which have not as yet been adapted to medical purposes. Many of these have industrial applications as preservatives or have been used in agriculture to control plant diseases. Table III shows some preliminary results with materials of this kind.

TABLE III

ASSAY OF BACTERIOSTATIC ACTIVITY
OF INDUSTRIAL COMPOUNDS

Density/cm.^{2} - Ten Subjects

Agent	Bacterial Density/cm.^{2}
Control	800,000
1% Arosurf	600,000
1% Hibitane Chlorhexidine	0
0.1% Hibitane	680
1% Tetramethylthiram	50
0.1% Tetramethylthiram	320
0.01% Tetramethylthiram	124,000
1% Cetyl piridinium chloride	300,000
1% Barquat	800,000
1% Dichlone	600,000
1% Piperalin	620,000
1% Chloranil	840,000
1% Dodecylquanine	660,000

II. Bacteriocidal Activity

It is apparent that chlorhexidine and tetramethylthiram disulfide were highly effective agents. Most of the remainder were unimpressive.

Substances may kill skin organisms efficiently, ethanol for example and yet, because they lack residual activity or are volatile, will fail in the bacteriostatic test. They might, nevertheless, be useful for certain applications such as sterilizing the skin prior to injection or biopsy. Cidal activity, therefore, must be measured by a different technique.

The procedure entails expanding the resident flora to a density of 10^5 organisms/cm.^{2} or greater by a 48-hour period of occlusion with impermeable plastic film. Saran wrap is wrapped around the entire length of the forearm in several layers and sealed at the ends with plastic adhesive tape for 48 hours. The Saran wrap is then removed and 5 cm. squares are marked out. The test materials are applied as in the bacteriostatic assay and the sites occluded for an additional 48 hours.

Examples of data secured in this assay are given in Table IV. It is clear at once that this is a stringent procedure. Cidal activity was generally evident only at the highest concentrations.

Table V shows the results obtained by this assay using proprietary products. Only the antibiotics fared well by this procedure. Vioform, Gantrisin, and 1% Haloprogin were not impressive.

TABLE IV

BACTERIOCIDAL ACTIVITY OF COMPOUNDS

Density/cm.^{2} - Ten Subjects

Agent	1% (40ug/cm.^{2})	0.1% (4ug/cm.^{2})	0.01% (4ug/cm.^{2})	Control
Bacitracin	15,000	26,400	107,400	2,090,000
Chloramphenicol	193	2,710	18,500	1,140,000
Chlortetracycline	21,200	165,000	532,000	377,000
Hexachlorophene	6,750	251,000	193,000	553,000
Benzalkonium chloride	18,800	640,000	477,000	307,000
Neomycin	1,270	9,250	103,000	954,000
Povidone iodine	1,630,000	1,420,000	1,191,000	1,940,000
Pyrithione sodium	1,230	31,900	335,000	677,000

* Bars encompass means not significantly different from one another.

TABLE V

BACTERIOCIDAL ACTIVITY OF PROPRIETARY PRODUCTS

Density/cm.^{2} - Ten Subjects

Agent	Bacterial count/cm.^{2}
Garamycin cream	569
Garamycin ointment	7,640
Neo-Cortef ointment (neomycin)	1,181
Neosporin cream	780
Polysporin ointment	4,500
Neosporin ointment	3,100
Archromycin ointment	3,480
Chloromycetin cream	9,450
Furacin cream	55,000
Povidone iodine (Betadine)	55,800
Vioform cream	118,000
Gantrisin cream	212,800
1% Haloprogin cream	450,000

The bacteriocidal test demands that an agent be able to destroy a teeming population of organisms but must also possess residual activity for 48 hours. This might be a requirement for a pre-surgical scrub when the operation site will be covered for a few days. Agents which do not pass this test may still have significant bacteriocidal activity. This requires a further test in which the time is greatly shortened. The forearm flora is first expanded by a 48-hour period of Saran wrap occlusion as above. The test agents are applied and occluded for only six hours before samples are taken. Carryover of the agent into the culture are minimized by the addition of lecithin and Tween-80 to the media.

This modification measures cidal activity alone. Typical results are shown in Table VI.

TABLE VI

SIX-HOUR BACTERIOCIDAL ASSAY

Density/cm.^{2} - 10 Subjects

Agent	6 Hours	Control	48 Hours	48-Hour Control
Betadine Ointment	400	400,000	858,800	900,000
0.1% Hexachlorophene	5,100	600,000	251,000	553,000
Vioform Cream	450,000	800,000	118,000	400,000
0.01% Neomycin	560	460,000	103,000	954,000

This shortened assay separates agents which have no bacteriocidal activity from those which have such an action but are without residual effect. Agents which pass this six-hour assay are effective but require more frequent applications and would not be appropriate in situations requiring prolonged activity such as under a dressing of a wound.

Both the bacteriostatic and the bacteriocidal tests are based on suppression of resident gram positive organisms, chiefly cocci and diphtheroids. Occasionally, overgrowth with gram negatives will give high counts with agents known to be effective, a false negative result. Indeed this eventually is only possible with agents that suppress gram positives. In reading the plates, therefore, one must ascertain the nature of the colonies being counted. Fortunately, gram negatives are easily recognizable.

III. Persistance Test

Agents which would be useful in the prophylaxis of cutaneous infections, in high risk situations (military activity, endemic ecthyma), should have the property of becoming bound by physico-chemical forces to the outer regions of the horny layer, that is, they should be substantive. Of perhaps even greater effectiveness would be materials that would diffuse into the horny layer, creating a reservoir; these might exert an antibacterial effect for several days after the last exposure.

The procedure is as follows: 5 cm. squares were marked out on each forearm. Each received 0.1 ml of a 1% aqueous or alcoholic solution of the test material three times a day for three successive days. Complete formulations such as creams may simply be rubbed over the site using the same schedule. The subjects were under no restrictions for the next three days. Washing with bland soaps was permitted. After three days, the squares were covered with the usual occlusive dressing for 24 hours. The center of the square was sampled immediately upon removal of the dressing.

Groups of 10 to 15 subjects, 172 in all, were treated with 1% solutions of 13 compounds. The results were analyzed by one-way analysis of variance, the ranked means compared by Tukey's procedure and allocated to "effective" or "ineffective" categories. Among antibiotics, chloramphenicol, neomycin, penicillin G, and gentamicin exhibited "substantivity" or a reservoir effect, whereas tetracycline, oxacillin and erythromycin did not. Among the chemotherapeutic compounds, only bronopol and sodium pyrithione persisted (TABLE VII).

Gram negatives (enterobacteria) were noted in 138 of the 341 samples but generally in small numbers, less than 5% of the microflora. They were more often encountered with agents classed as ineffective, 81 of 169 (48%), compared with effective ones, 57 of 172 (33%) (x^2=7.14; $P<0.01$). Ineffectiveness, however, could not be attributed to overgrowth by gram negatives as can occur in the expanded flora test.

TABLE VII

PERSISTANCE TEST (Substantivity)

Ten Subjects

Material	Density/cm.^{2}
Chloramphenicol	4,130
Neomycin	5,250
Bronopol	13,270
Penicillin G	21,800
Sodium pyrithione	25,350
Gentamicin	35,800
Tetracycline base	118,600
Sulfamylon	192,000
Hexachlorophene	263,000
Hyaminel 10X	468,000
Oxacillin	697,000
Erythromycin	1,480,000
Hexetidine	1,820,000

SUMMARY

We have attempted to develop simple methods for ascertaining the capacity of substances to exert material antibacterial effects when applied to human skin. It will be seen that we have employed several variations of a single tactic, namely, that of stimulating the multiplication of resident micro-organisms by rendering the surface moist with an occlusive dressing. A minimum requirement of a topical agent is the ability to curtail microbial growth in the presence of secretions (sweat, sebum) and surface constituents (mainly nitrogenous materials). One can distinguish bacteriocidal from bacteriostatic effects and evaluate the duration of the suppressive effect. Moreover, these effects are quantifiable.

Further study utilizing these techniques is in progress to establish assays for determination of 1) an agent's propensity for inducing ecological shifts, i.e., encouragement of a gram-negative flora or an overgrowth of yeasts, 2) ability of an agent to demonstrate antibacterial activity in different body regions, e.g., areas of high sebum where the potential problem of inactivation by lipids exists, activity in excessively moist areas such as the axilla and toe web space where dilution effects may occur.

Paired Comparison Studies with Topical Antibiotics

There is now sufficient evidence to indicate that application of an active antimicrobial agent to one part of the body can result in enough translocation of that agent to affect the density of organisms on other body areas. This limits the usefulness of paired comparisons in which the subject serves as his own control.

Neomycin cream (1%) versus Placebo in chronic dermatoses

A double blind study was conducted in 24 patients with chronic dermatoses including 11 in whom lesions were colonized by *Staphylococcus aureus*. The underlying diseases were atopic dermatitis, psoriasis and infected eczematous conditions. Each patient received two coded medications to be applied to symmetrical lesions once daily for seven days. The patients were shown how to apply the materials so as to avoid cross-contamination. Each was told that two treatments were being studied to determine which was most suitable for the individual and encouragement was given to ensure proper application. The number of aerobic organisms and of *S. aureus* was determined before and after treatment by the quantitative detergent-scrub technique.

Unexpectedly, especially in those harbouring large numbers of *S. aureus*, clinical improvement of an appreciable degree was noted on both sides. The lesions, of course, did not disappear but were clearly less influenced. Indeed, several areas that received no application whatever improved. After the code was broken, it was evident that improvement was greater on the neomycin sites, but the clinical benefit in other diseased areas was nonetheless clearly discernible.

The microbiological data provided an explanation of the clinical findings. Firstly, the total number of aerobic organisms fell greatly on both sides, from a mean of 11,900 to 405/cm.^{2} in the neomycin sites and from 12,900 to 2275 for the control. Though the percentage reduction was greater with neomycin, a decrease of more than 80% at placebo sites is an impressive change. Secondly, neomycin completely eliminated carriage of S. aureus in six of eleven patients and another two showed a reduction of more than 99%. In this respect, effectiveness of the placebo was almost as great, five sites showing complete eradication of the pathogen and two a 99% reduction. In only one placebo site was more S. aureus recovered after than before treatment (TABLE VIII).

These results might be explained in two ways. Either the placebo was antibacterial or neomycin had spread over the surface from the site of application. The first possibility was ruled out when a further six patients whose lesions were heavily colonized with S. aureus were treated with only the placebo cream for seven days and then subsequently with neomycin alone for a week. After 1 week of placebo treatment there was no change in the density of micro-organisms (TABLE IX). Subsequent therapy with 1% neomycin essentially sterilized the sites. This experiment demonstrates that the reduction of organisms at the placebo-treated sites in the first experiment (TABLE VIII) was due to the spread of neomycin.

TABLE VIII

1% NEOMYCIN CREAM VERSUS PLACEBO

(density of S. aureus/cm.^{2})

Neomycin		Placebo	
Pre-Treatment	Post Treatment	Pre-Treatment	Post Treatment
852,000	21	947,000	8
458	0	144	0
8,170,000	3,650	3,050,000	0
1,200	105	408,000	0
4,470	842	3,420	66
92	0	211	0
2,470,000	0	10,800,000	66
83,700	0	47,000	0
5,260	0	11,000	4,210,000
431,000	84,200	1,750,000	421,000
94,700	0	1,180,000	189,000

TABLE IX

Density of *S. aureus*/cm.^{2} on atopic skin after successive one week periods of treatment first with placebo, then with 1% Neomycin cream.

Pre-Treatment	Placebo	1% Neomycin
763,000	842,000	0
253,000	1,890,000	0
201,000	132,000	126
1,240,000	842,000	0
3,160,000	2,680,000	0
211,000	66,000	132
970,000	1,080,000	43

The placebo did not lower the bacterial population while subsequent applications of neomycin drastically reduced the counts.

Effect of 1% Topical Penicillin G on axillary flora

Because of warmth and moisture the axilla supports a huge number of resident organisms usually exceeding a million/cm.[2] We found previously that the most sensitive indicator of the arrival of an orally administered antibiotic at the skin surface was an increase in the proportion of cocci resident to the antibiotic. This change in the resistance pattern occurred without a decrease in the total number of organisms. For example, when demeclocycline was administered for three weeks, coccal resistance rose from 10 to 90% within the first week. With clindamycin coccal resistance rose from undetectable levels to 80% within a three week period. In these incidents, the initially susceptible organisms were killed by the low surface levels of the antibiotic enabling resident cells to multiply till the original population was restored.

Five healthy volunteers received 1 ml. of a 1% aqueous penicillin G to one axilla once daily for two weeks. The opposite axilla was not treated. Samples were taken from both axillae before, and after one and two weeks of treatment. Resistance was determined by the disc method.

There was no change in the total number of organisms in either axilla. With regard to coccal resistance, however, striking alterations occurred in both (TABLE X). By the second week of treatment nearly all of the cocci were resistant to penicillin in both axillae. It seemed very likely that small amounts of penicillin had migrated to the untreated axilla inducing therein a resistant population. Mechanical transfer of resistant cells from the treated axilla is ecologically an unlikely explanation of the findings since in the absence of the drug the tendency is for the population to lose resistance.

TABLE X

Effect of topical penicillin on percentage of resistant cocci in axill

	Untreated Control		Penicillin	
Subject	Pre-Treatment	2 Weeks	Pre-Treatment	2 Weeks
1	15	90	15	100
2	75	100	75	100
3	85	100	85	100
4	5	70	5	*
5	5	100	5	100

*Too few cocci recovered for testing.

Assay of Bacteriostatic Soaps in the Axilla

The effectiveness of bacteriostatic soaps is often appraised in handwashing tests; these give highly variable results. The bacterial population is low to start with and the organisms are very unevenly distributed. We utilize the axilla as a model because a mixed flora occurs there and the quantity of organisms is very high. The area is also rich in skin secretions, adding rigor to the test.

Deodorancy is a crude criterion of antibacterial efficacy. Quantitative bacteriologic study is much more reliable. We did two studies: 1) a 2% hexachlorophene soap was compared to a non-medicated soap and, 2) a 2% hexachlorophene soap was evaluated against one containing tribromosalicylanilide and halogenated carbanilides. Opposite axillae were lathered and rinsed twice daily for ten days. Microbial samples were obtained before treatment and on the morning after the last washing.

In the first study, the hexachlorophene soap reduced the bacterial population by 72% in comparison to only 19% on the control side, a highly significant difference. This result is straight forward and non-provocative. However, the same hexachlorophene soap behaved quite differently in the second study. This time there was a reduction of 96% which was not significantly different from the opposite side treated with a potent bacteriostatic soap.

Our explanation is that 2% hexachlorophene soap was only moderately active. In the first study it did not appreciably affect the organisms in the control axilla. In the second study, however, translocation from the axilla treated with a more powerful antimicrobial soap effecting virtual obliteration of the flora on the opposite axilla, conferring a specious potency on the hexachlorophene soap. We have encountered this phenomenon several times. After ten days of use of a potent bacteriostatic agent in one axilla, a comparable reduction in the bacterial count occurs in the opposite one whether it is untreated or washed with a non-medicated soap.

These studies again demonstrate translocation but they also illustrate the suitability of the axilla for assaying bacteriostatic detergents or indeed any topical formulation. A small number of gram negatives is often found in the axilla. These can become dominant when the gram positives are eradicated. The axilla therefore is an appropriate place to detect ecologic shifts from one-way bacteriostats.

TABLE XI

Comparisons of the Antibacterial Effects of Bacteriostatic Soaps in Opposite Axillae

	First Study (41 Subjects)		Second Study (40 Subjects)	
	2% Hexachlorophene	Non-medicated	2% Hexachlorophene	TBS + carbinilides
Pre-Treatment	1,518,000*	1,840,000	1,404,000	1,419,000
Post-Treatment	424,000	1,484,000	61,500	39,900
% Reduction	72	19	96	97
Reduction from original level	s**	ns	s	s
Comparison of reduction	s		ns	

* Total aerobes/$cm.^2$ (geometric mean)
** Significant, $P<0.01$

Spread of Antimicrobial Agents from Patch Tests

These studies were concerned with estimating the length of time an antimicrobial agent could continue to have a repressive effect on the microbial flora at the site of the application. This is actually a modification of the persistance test. The design was to apply a thin film of a 5% concentration of the test agent in hydrophilic ointment USP to a 5 cm. square on the volar forearm. The area was immediately covered with a 5 cm. square of polyethylene film and sealed with impermeable plastic tape for 24 hours. The site was rinsed with water after removal of the dressing. The subjects were then permitted to wash as they pleased for the next three days. On the fourth day, the treated site and a control site on the opposite forearm were covered with polyethylene occlusive dressing for 24 hours, a procedure which hydrates the skin and induces the multiplication of resident organisms to densities in excess of 10^6/cm.[2]

The results were unexpected. Agents were inhibiting multiplication of the resident flora on the control, untreated site. The two most impressive examples were obtained with chloramphenicol and sodium pyridine-thione. As shown in Table XII, the organisms in the untreated sites failed to reach the expected levels, being less than 10^4 in five of eight sites. By contrast, the counts exceeded 1×10^6/cm.^{2} in all but one of the controls with erythromycin and tricholoro-2-hydroxy-diphenyl ether (Irgasan CH 3565 - Geigy Chemical).

An idea of the amount transferred can be gained by determining the least quantity of chloramphenicol required to prevent multiplication under occlusion. Serial, ten-fold dilutions of this antibiotic were applied to the skin and immediately covered with an occlusive dressing for 24 hours. It was found that as little as 4 $mg/cm.^2$ kept the microflora from increasing appreciably.

This experience again demonstrates the difficulties one can innocently fall into by not taking into account the possibility of transfer of substances over the skin surface. This is a handicap of paired comparison studies only with substances which exert powerful effects in minuscule amounts. Antibiotics are the best examples so far. The literature contains several instances in which failure to approach translocation led to false conclusions. With steroid-antibiotic combinations evaluated blind in paired comparisons to steroid alone or opposite sides of chronic dermatoses, a virulent organism was suppressed in both sides and improvement was similar. The conclusion was that antibiotic-steroid combinations have no merit over steroids alone in treating infected dermatoses. This is a specious interpretation and may have the consequences of removing such combinations from the market.

TABLE XII

TRANSLOCATION FROM PATCH TESTS

(Bacterial density after 24 hours' occlusion

and a three-day free period)

Agent	Treated (Count/cm.^{2})	Untreated (Count/cm.^{2})
Chloramphenicol	340	6,800
	30	320
	60	3,600
	30	80
Sodium pyridine-thione	10	2,080,000
	10	192,000
	2,400	60
	32,000	2,400
Erythromycin	8,800	3,200,000
	960,000	1,320,000
	5,200,000	64,000,000
	36,000	720,000

S. aureus in Chronic Dermatoses

It has long been appreciated that virulent staphylococci readily colonize dermatitic skin. Our concern has been to quantify the organisms in the lesions and on uninvolved skin. We sought also to establish criteria to judge when colonization is of such intensity as to warrant a diagnosis of secondary infection. Data of this kind have epidemeologic importance since shedding of bacteria lacks scales from chronic dermatosis may be a public health hazard.

Psoriasis

Our investigations had the following objectives: 1) to determine the quantity of S. aureus on psoriatic plaques and uninvolved skin, 2)to characterize quantitatively the aerobic microflora of the skin of psoriatics, and 3) to assess the effect on the bacterial flora of occluding psoriatic skin with impermeable plastic film, a technique often used to enhance the penetration of drugs.

Patients

92 lesions in 61 patients were studied. In 50, both lesional and non-lesional skin were sampled. The uninvolved site (normal) was on a comparable area on the opposite side or at least five cm. from the edge of the plaque. The subjects were chiefly out-patients, or in a few instances, newly admitted, to avoid the problem of hospital acquisition of S. aureus. They were not on treatment at the time of sampling. Psoriatic erythroderma was excluded. Plaques sampled were on non-specialized glabrous skin avoiding the scalp and intertriginous regions.

Effect of Occlusion

A plaque of psoriatic skin and a comparable normal site in 14 patients were covered with an occlusive plastic film dressing, 5 cm. squares, held firmly in place with impermeable adhesive tape for a week.

Sampling Method

Quantitative samples were taken by the detergent scrub technique. A glass cylinder 3.8 sq. cm. in area was held to the skin and the surface rubbed firmly with a Teflon rod in 1 ml. of wash fluid (octyl-phenoxy polyethoxyethanol - Triton X-100) in phosphate buffer for one minute. The fluid was removed and the procedure was repeated. Serial ten-fold dilutions were plated as single drops on trypticase soy agar (TSA) and TSA with lecithin and polysorbate 80 (Tween 80). Streak plates were made on Marshall and Kelsey agar. Total density and density of each major microbial group was expressed as organisms per square centimeter. A logarithmic transformation was applied before statistical analysis.

Aerobic Bacterial Density

The number of bacteria on involved and uninvolved skin varied tremendously. On normal skin, the counts ranged up to 48,000 with a geometric mean only 311 organisms per square centimeter. The low geometric mean was due to an excess of low counts, perhaps reflecting the use of antibacterial soaps. The modal value of 10^3 organisms per sq. cm. closely resembles that found for glabrous skin on the forearm.

The geometric mean count on psoriatic skin was 1,840 sq.cm., a moderate but statistically significant increase over normal skin ($t=3.97$, $P<0.01$). The distribution was closer to log normal than the above data, but still somewhat skewed by a few abnormally low counts. The quantities on normal and diseased skin paralleled each other (52 pairs $r=0.51$, P 0.01).

Staphylococcus aureus

Nearly half of the psoriatic lesions were colonized by *S. aureus* (42 of 92, 46%). Normal skin yielded *S. aureus* in only 14 of the 52 sites (27%), significantly fewer ($x^2=4.15$, $P<.05$), but still many times greater than on normal persons. In all but one patient, *S. aureus* was recovered from the lesion when the clinically normal site was positive. The densities on normal skin were mostly low, with a geometric mean of 103. Again, the counts on normal and diseased skin were parallel. The density of *S. aureus* on psoriatic skin ranged from 20 to 300,000 per sq.cm. with a geometric mean of 928 sq.cm. Eight lesions (9%) carried in excess of 10^4 *S. aureus* per sq. cm.

Composition of Aerobic Microflora

Essentially all samples of diseased and normal skin contained moderate numbers of a coagulase negative coccus(TABLE XIII). Lipophilic diphtheroids were isolated from two-thirds of the lesions and over half the normal sites. Other diphtheroids and gram-negative rods were present in low numbers, most often in lesional skin. The remainder of the flora comprised yeasts with occasional isolation of a few other organisms, including true fungi, Bacillus and other less well characterized groups.

TABLE XIII

Incidence of Bacterial Groups on Psoriatic Plaques and Uninvolved Skin

	Lesions		Normal	
Organisms	No.(92)*	%	No.(52)*	%
Coagulase-negative cocci	90	98	45	87
S. aureus	42	46	14	27
Lipophilic diphtheroids	62	67	27	52
Other diphtheroid	17	18	8	15
Gram-negative rods	12	13	4	8
Yeasts	5	5	2	4
Other	5	5	1	2

* Numbers in parentheses indicate totals.

Occlusive Dressings

After continuous occlusion for one week, the density of bacteria on psoriatic plaques increased from thousands to tens of millions per sq. cm. When S. aureus was also present, the number increased enormously, up to 100 million per sq. cm. in one case. The density on the normal site rose from 404 to 825,000 organisms per sq. cm. Staphylococcus aureus was present in six samples from normal skin before occlusion and in seven post occlusion. The mean density in carriers rose from 185 to 61,5000 S. aureus per sq. cm.

Before occlusion, the mean on psoriatic skin was 1,020 organisms per sq. cm. Afterwards, the numbers rose to 2.87×10^7 a considerably greater increment than on occluded normal skin ($t=3.30$, $P<.01$).

Staphylococcus aureus was recovered from eight lesions before occlusion and nine after occlusion. The mean density increased from 1,440 to 11,650,000 S. aureus cells per sq. cm. In one patient, the S. aureusdisappeared and two acquired the organism. In subjects whose counts became extremely high, the lesion became redder and more edematous though not obviously infected by the usual criteria.

Atopic Dermatitis

70 patients ranging in age from four months to 19 years were studied. The majority were children between two and ten years, with a prolonged history of the disease. In 50, the lesions were typical, pruritic, chronic, lichenified, non-exudative plaques of atopic dermatitis. The antecubital fossa was the site usually sampled. 62 lesions in these 50 patients were evaluated in a quantitative fashion

for aerobic bacteria. In 27, clinically normal skin 5 cm. or more away was similarly sampled. At the time of sampling, none was using bacteriostatic soaps, or topical or systemic antibiotics. In another 20, acute exudative lesions were sampled during an acute exacerbation of the disease. Again, the majority of lesions sampled were in the antecubital fossa. The afflicted skin showed erythema, oozing, microvesiculation and occasionally slight crusting. Frankly infected lesions were not sampled. No patient had fever, lymphadenopathy or leukocytosis. In 14 of this group of patients, clinically normal skin 5 cm. away was also sampled.

Bacteriological samples were obtained by the detergent scrub technique. Single drops from a tenfold dilution series were plated on (1) Trypticase Soy Agar (TSA), a non-selective medium, and (2) TSA with lecithin as a detoxicant and with polysorbate 80 to enhance growth of S. aureus. In addition, streaks were made on Marshall and Kelsey's medium. The plates were incubated aerobically at 35° C for two days. The total aerobes per sq. cm. and the proportion comprised by S. aureus were then determined.

13 of the patients with chronic lichenified lesions were treated with a 1% Neomycin cream twice daily for seven days, followed by bacteriological evaluation on the eighth day. Another 11 received Erythromycin, 250 mg. four times daily. We sought primarily to compare oral and topical treatment with respect to elimination of S. aureus. Additionally, we examined the possibility of relating antibacterial efficacy to clinical response. The effect of treatment was evaluated

by observing changes in erythema, lichenification and excoriation. Using these indicators, the response was designated as "improved, no improvement or worse." No other therapy was given during the one week of antibiotic therapy.

Cultures from Dermatitic Skin

In chronic, lichenified plaques the mean density of 66 samples was 593,000 organisms per sq. cm. *S. aureus* was recovered from 60 sites (91%) with a mean density of 403,900 /cm.^{2} In the 20 acute, exudative lesions, the mean density of aerobes was 18,417,000/cm.^{2} *S. aureus* was recovered in all 20 (100%) with a mean density of 15,122,000/cm.^{2} In both types of lesions, therefore, *S. aureus* was overwhelmingly the predominant organism. In chronic plaques, pure cultures of *S. aureus* were obtained in nine patients; the density exceeded 10^6 cm.^{2} in 31 (45%). In the exudative lesions, ten patients yielded pure cultures of *S. aureus*; thus exudative lesions carried a tremendously greater number of total aerobes ($t=3.77$, $P<0.01$) and of *S. aureus* ($t=3.33$, $P<0.01$).

Comparison of Normal and Dermatitic Skin

S. aureus was isolated from all 15 paired cultures of normal and dermatitic skin in the patients with exudative lesions, in 21 out of 27 cultures of normal skin, in 24 out of 27 cultures from the chronic lichenified plaques. This difference in prevalence was not significant. The density of *S. aureus*, however, was very much greater in dermatitic skin. Lichenified plaques had a mean density of 554,300 *S. aureus* per cm.^{2}, compared to only 1,358/cm.^{2} on normal skin ($t=6.23$, $P<0.01$%).

Likewise, the total aerobic flora was considerably larger in dermatitic skin, 718,900 against 2624 cm.^{2} ($t=10.5$, $P<0.01\%$). In patients with exudative lesions, a mean density of 14,505,000 *S. aureus* per cm.^{2} was found, compared to a mean density of 182,352/cm.^{2} on normal appearing skin ($t=8.8$, $P<0.01\%$). Likewise, the total aerobic flora was again considerably larger in dermatitic skin, a mean density of 16,646,428/cm.^{2} in lesions compared to 232,307/cm.^{2} in normal skin ($t=9$, $P<0.01\%$).

Effect of Antibacterial Treatment

All 13 patients treated with neomycin cream twice daily had *S. aureus* prior to treatment, the mean density being 360,000/cm.^{2} Eight had 1,000,000 or more organisms per cm.^{2}; the mean was unduly lowered by four patients who had only 200,000 organisms per cm.^{2} or less. After one week of neomycin, *S. aureus* was completely eradicated in six. In the remaining, the mean density was a mere 388/cm.^{2} In the eleven treated with oral Erythromycin, the mean density of *S. aureus* before treatment was 1,700,000/cm.^{2} After a week of therapy, *S. aureus* was totally eradicated in three and the mean count was 1890/cm.^{2} in the rest. The total aerobic count was reduced from 3,334,000 to 6,180/cm.^{2}

SUMMARY

These two studies demonstrate that inflamed skin frequently becomes colonized with S. aureus. In atopic dermatitis, the lesions are wetter with more serious leakage, and the density of S. aureus is thus much greater than in psoriasis. In both conditions, however, S. aureus can be of significance to the patient and his environment. In atopic dermatitis, 45% of indolent lesions are colonized by more than a million S. aureus organisms per sq. cm. while exudative but clinically non-infected lesions harbor tens of millions of this organism. Antibiotic therapy, topical or systemic appears to be beneficial in promoting swifter response to therapy. In psoriasis, S. aureus levels rarely reach the harmful level seen in atopic dermatitis except under the stimulus of occlusive dressings; steroids are often applied in this fashion. Proliferation of S. aureus under occlusion, sometimes accounts for sluggish response to therapy or even complete resistance. Both psoriasis and atopic dermatitis heavily colonized with S. aureus can represent major public health hazards. In both conditions, there is an accelerated desquamation of stratum corneum cells which can serve as a source for cross infections. In fact, psoriatic skin has been identified as the source of serious cross ward infections due to S. aureus

We are planning to compare the effects on the microflora of occlusive application of steroid and steroid-antibiotic combinations. This will be completed with clinical responses.

Topical Steroid-Antibiotic Combinations in Experimental Infections

There is much debate whether steroid-antibiotic combinations are rational and justifiable. Clinical studies have come to opposite conclusions. An important and virtually uncontrollable source of difficulty is patient variability. Moreover, it is not at all easy to determine on clinical grounds whether lesions carry large or small numbers of virulent organisms. We have turned to experimental models to resolve certain aspects of the problem.

Subjects

The subjects were young, adult prison volunteers. They were judged to be in good health by examination, history and normal values for the following laboratory tests; complete blood cell count, serum glutamic oxaloacetic transaminase, blood urea nitrogen, fasting blood glucose and urinalysis.

Materials

The test materials were: 1) complete formulation, 0.1% triamcinolone acetonide, 100,000 units/gm nystatin, 0.25% neomycin, and 0.25% gramicidin (Mycolog cream): 2) triamcinolone acetonide, 0.1% in the cream base (Kenalog cream): 3) nystatin cream 100,000 units/gm (Mycostatin cream); 4) neomycin, 0.025% and gramicidin, 0.025% (Spectrocin cream): 5) triamcinolone acetonide 0.1%, neomycin, 0.25% and gramicidin, 0.025% (Kenalog S); and 6) a cream base.

Experimental Models

S. aureus Infections

The method for inducing cutaneous *S. aureus* infection has been previously described. Six 2-cm. squares were marked out on the volar surfaces of the forearms, three per forearm, in 13 volunteers. After delineating the squares with adhesive tape, the horny layer was removed down to the "glistening layer" with cellophane tape (Scotch tape). The stripped sites were left exposed for the next 24 hours.

The inoculum was prepared by diluting hundredfold a "just hazy" saline suspension of a strain of S. aureus sensitive to the ordinary antibiotics. A volume of 10 µl was applied to each square with a capillary micropipette. An occlusive dressing was immediately applied, consisting of a 2-cm. square of plastic film (Saran wrap) and overlapping strips of cloth-backed adhesive tape. Six hours later, the bandages were removed and approximately 30 mg. of cream delivered to each site directly from the tube. This quantity amply covered the surface when spread out under a 2-cm. square plastic film. The occlusive dressing was then reapplied for another 18 hours. The creams were coded according to the double-blind format and the sites randomized in each subject.

The effect of treatment was evaluated in three ways: (1) Quantitative bacteriological analysis with the detergent scrub technique was used to estimate the density of S. aureus; (2) Cytologic analysis was done on the exudate by pressing a glass slide to the under surface of the plastic film. The quantity of neutrophils was estimated on a 0 to 4 scale in which 0 signified virtual absence of leukocytes and 4 solid massing; (3) Clinical severity was assessed on a 0 to 4 scale in which the highest value represented purulent erosion and the lowest merely the effect of stripping, comparable to an uninoculated site.

Candida Albicans Infections

This procedure has been detailed previously. A suspension of freshly grown C. albicans cells was made in saline solution and counted in a hemocytometer. Appropriate dilutions were made to provide inocula containing 10^3, 10^4 and 10^5 cells per 10 ul.

Twenty 2-cm. squares were marked out in 20 volunteers in five columns of four sites on the forearms, three on one forearm, two on the other. In each column, one site received no inoculum, one 10^3, one 10^4, and one 10^5 C. albicans in 10 µl volumes from capillary pipets. Approximately 30 mg of cream was then delivered to each site which was immediately covered with a 2-cm. square of plastic film and sealed occlusively to the skin for 24 hours under adhesive tape. Coded treatments were applied in a randomized fashion. The sites in one column received no cream and served as a non-treatment control for each dilution. The antibacterial formulations were not studied in this yeast infection. The sites were sampled at 24 hours by the detergent scrub technique to determine the density of C. albicans. In an additional 24 hours (48 hours post inoculation), the severity of the infection was assessed on the following scale: 0, no lesion; +, 1 to 5 vesicles; ++, 5 to 20 vesiculopustules; +++, dense pustules; ++++, suppurative bullae; +++++, erosions.

Microbiological Methods

These followed our published procedures. To sample the site, a glass cylinder was held to the skin and the surface was rubbed with

with 1 ml. of phosphate-buffered detergent (0.1% octylphenoxy polyethoxy-ethanol [Triton X-100]) with a blunted Teflon scrubber. Two such washes were pooled from which serial t nfold dilutions in detergent were made. Drops were placed on trypticase soy agar with or without lecithin and polysorbate 80 to estimate total numbers of aerobes. Polymyxintetrazolium medium was used for S. aureus and Mycosel and Sabouraud dextrose agars for C. albicans. The plates were incubated for 48 hours.

Results

S. aureus Infections

The results are shown in Table XIV. The severity of the reaction was the same whether judged clinically or by examination of the exudate. The untreated sites were, as expected, the worst. Although the cream base did not interfere with the growth of S. aureus, the inflammatory reaction was somewhat less than in untreated sites. The steroid alone clearly moderated the lesion, even though the multiplication of S. aureus was not impeded, the count being 40 million per sq. cm. compared to 25 million per sq. cm. in the control site. With neomycin-gramicidin, the count fell drastically to an average of 20,000 per sq. cm. No S. aureus could be recovered in eight of 13 sites. Formulations containing these antibiotics were always associated with very low counts and the infections were correspondingly mild. The organism did not survive at all in 25 of the 39 sites receiving antibiotics alone or in combination. The addition of tramcinolone to the antibiotics almost completely suppressed the signs of infection. As expected the presence of nystatin (the complete formulation) did not change this outcome.

TABLE XIV

Effect of Antibiotic-Steriod Combinations on S. aureus Infections

Treatment	Density of S.aureus	Organism Absent (13 sites)	Mean Clinical Score	Mean Cytologic Score
Triamcinolone, neomycin nystatin, gramicidin	15,000	7	0.77	1.08
Triamcinolone, neomycin gramicidin	80,000	10	0.85	0.92
Neomycin-gramicidin	20,000	8	1.15	1.17
Triamcinolone	40,000,000	1	2.15	2.33
Cream base	24,000,000	0	2.31	2.85
Untreated	25,000,000	0	3.00	3.31

Candida albicans Infections

The results obtained with the 10^4 inoculum will illustrate the effects of the various combinations (TABLE XV). In this case, the presence of nystatin eradicated the test organism completely; naturally no lesions developed. Again there was a modest clinical improvement with the cream base alone despite a somewhat greater growth of *C. albicans*. With steroid alone, the density of *C. albicans* was identical to that of the untreated site, yet the severity of the pustular reaction was greatly diminished. The complete formulation was, of course, no more effective than nystatin alone, the pathogen having been killed in both instances. The pattern was very much the same with the other inocula; *C. albicans* density and the severity of the lesions in untreated sites was greatest with 10^5 and least with 10^3.

TABLE XV

Effect of Antibiotic-Steroid Combinations on *C. albicans* Infections

(Inoculum 10^4)

Treatment	Density of *C. albicans*	Organism Absent (20 sites)	Mean Clinical Score
Triamcinolone, nystatin, neomycin, gramicidin	0	20	0
Nystatin	0	20	0
Triamcinolone	16,630	0	0.425
Cream base	29,560	0	1.10
Untreated	16,470	0	2.23

GENERAL CONCLUSIONS

Experimental models are, of course, contrived to enable specific items to be analized under controlled conditions. The infections we created are not isomorphic with secondarily infected chronic dermatoses. Still some useful information was given which may be summarized as follows:

(1) Steroids alone moderate the clinical signs of bacterial and yeats infections even though the organisms multiply to very high levels. The fear of severer infection through interference with host defences, a definite phenomenon with systemic infections, was not realized. By suppressing the inflammatory response provoked by the organisms, the lesions were muted. Exudation diminishes and the surface environment becomes less hospitable to the growth of organisms. While steroids alone would in time doubtless result in restoration to normal, large numbers of pathogens would continue to be dispersed into the environment. We found in preliminary studies that S. aureus was plentiful even when healing was nearly complete.

(2) Antibiotics alone usually eradicated the pathogenic agent and, of course, reduced the signs of infection.

(3) The swiftest and clearest suppression was secured with the antibiotic-steroid combination. This could hardly be otherwise; the organisms were killed and the reaction induced by their toxins suppressed.

We have extended these studies to an evaluation of steroid-antibiotic combinations in S. aureus-colonized chronic dermatoses. Because of translocation only one treatment per patient can be allowed. Using bacteriologic and clinical criteria we have provisional evidence that the combinations are superior to the individual components.

Experimental Candida Infections

The models we have described in the literature are acute pustular reactions which do not resemble clinical monialiasis. To study properly the dynamics of Candida infection, especially interactions with bacteria, requires a more realistic model. We were successful in reproducing in every detail erosion interdigital blastomycetia of the finger web.

Material and Methods

The subjects were 53 male prisoner volunteers (18 blacks and 35 whites) who ranged in age from 21 to 59 years of age. Good health was ascertained by physical examination and routine blood and urine studies. 24-hour subcultures of C. albicans on Sabouraud medium were used. The yeast cells were suspended in saline and counted in a white blood cell hemocytometer. Ten microliters of a suspension containing 10^5 cells was placed on the web between the third and fourth fingers. The two fingers were immediately bound together at the first joint with one-half inch plastic tape. This completely obliterated the interspace but still enabled the fingers to bend.

The contralateral interdigital space was similarly occluded but not inoculated.

The tape was reapplied every few days whenever it seemed to be loosening. The microflora was sampled by thoroughly swabbing the interspace with a cotton-tipped applicator wetted with 0.1% octylphenoxy polyethoxyethanol (Triton X-100). Appropriate dilutions were plated onto the following media: Trypticase soy agar, with and without lecithin and polysorbate 80; casein yeast extract lactate glucose agar, which is a rich general purpose medium; MacConkey's agar for the isolation of coliform and enteric organisms; phenlethanol agar for gram-positive rods, and Mycosel for _C. albicans_. Colonies were counted after two days at -37° C.

Results

Evolution and Morphologic Characteristics

The lesions began to develop within three to four days. Takes occurred in 80% of white subjects but none of the 18 blacks. Subsequently, we were careful to secure occlusion by using a splint beneath the fingers along with adhesive tape wrapping. With this manoeuver, we succeeded in creating EIB in four of six blacks.

The mature experimental lesion mimicked the picture of the native disease. The subjects bore the lesion with ease, complaining only of pruritus. The central area of the web became whitish and macerated with shreds of separated tissue. Along the inner sides of the fingers, erythema and pustules could often be seen. Later on, the horny layer separated, leaving a bright-red eroded surface that is so characteristic of the disease. This appearance persisted as long as the tape was kept on. Removal of the dressing led to prompt regression, with healing nearly complete within about a week. Occasionally, scaling and itching persisted for several weeks. The control side showed only whitish color maceration.

Microbiologic Findings

The microbiological data for severely inflamed interspaces are shown in Table XVI. Aerobes were greatly increased averaging about 3.8 x 10^7. This differed significantly from 1 x $10^{5.7}$ organisms for the control site. Without occlusion, the microbial population in this region generally does not exceed several hundred cells. The numbers of diphtheroids largely depended on the large colony diphtheroids, which although absent in the control areas, increased with time progressively. Lipophilic diphtheroids showed a general tendency to increase.

Gram-negative rods increased precipitously in the first five days, practically replacing the cocci; thereafter, they proportionately diminished to about a quarter of the population. Lactose fermenters decreased with time, being replaced by non-fermenters. Among occasional isolates were Pseudomonas, Proteus, Escherichia, and Mima-Herellea; colonization was occasionally limited to one of these.

Surprisingly, the percentage of the toal flora made up by C. albicans was extremely low throughout the study, never exceeding 1%. Their actual number, of course, was rather great, 1 x 10^5 at least. C. albicans was recovered in only one of three cases at 30 days.

CONCLUSIONS

Some valuable insights were derived from this data. Firstly, it is possible when circumstances permit to create infections safely which mimic the native disease. This is invaluable for studying factors which influence its course and for assessing the effectiveness of therapy.

Secondly, skin infections are rarely "pure." Streptococcal ecthyma is so often complicated by *S. aureus* that students have stridently quarrelled which one actually incited the disease. Indeed, *S. aureus* arrives later and may displace the streptococcus. Likewise, we actually found in thirty-day old infections that *C. albicans* could no longer be recovered in about 15% of cases. It never in fact made up more than 2% of the total flora. What is important is the absolute number which was often of the order of 10^5. Another finding was the constant presence of gram negatives. This suggests that moniliasis is more than a pure fungus infection. The great likelihood is that bacterial-fungal synergism is involved. An association between *Candida* and gram negatives has been observed in diaper rash, erosio interdigitali, onychomycosis and other disorders. They, therefore, may have to take into account the entire microbial population. The ancient advice of managing moniliasis by "drying" may actually be directed to gram negatives which require wetness to live.

TABLE XVI

PREVALENCE OF YEASTS AND AEROBIC ORGANISMS

IN EXPERIMENTAL EROSIO INTERDIGITALIS BLASTOMYCETICA

Days	Number	Number of Spaces Yielding				Total Aerobes
		C. albicans	Gram-Negative Rods			
			Total	Proteus	Pseudomonas	
5	10	10 (0.07%)*	7 (61%)*	2	1	$10^{6.9+0.3}$
12	9	9 (0.16%)	9 (16%)	1	1	$10^{7.1+0.3}$
15	7	6 (0.73%)	7 (23%)	1	1	$10^{6.7+0.4}$
30	3	1 (0.03%)	2 (33%)	0	0	$10^{7.5+0.1}$
15 (control)	7	0 (0.00%)	3 (0.3%)	1	1	$10^{5.7+0.6}$

*Percentages in parentheses are of the total.

"ATHLETE'S FOOT SYNDROME"

In a previous report, we indicated that the clinical and microbiological finding of interdigital "athlete's foot" varied from a dry scaling variety due to dermatophytes or C. minutissimum to a macerated heaped-up, leukokeratotic variety in which dermatophytes may or may not be present and in which aerobic diphtheroids and sometimes gram-negative organisms are plentiful. In our view, the dry, scaling type is a simple dermatophyte infection. It is usually asymptomatic and is usually ignored. The "wet" type is a collaborative enterprise between bacteria and fungi and is generally asymptomatic. It is often accompanied by pruritus, tenderness, maceration and foul odor. The interspace is soggy and white, with variable scaling. Fungi initiate the lesion but are often absent during exacerbations. This variety in our experience responds poorly to the available commercial agents including tolnaftate, haloprogin and miconazole. These agents are primarily antifungal and possess only limited antibacterial activity. In addition, these agents do not provide any "drying" or astringent action which is so necessary for successful therapy of a wet, macerated interspace. In these experiments, we evaluated the efficacy of various aluminum salts which have both an antibacterial action as well as an astringent effect.

Antimicrobial Activity of Aluminum Salts

I. Bacteriostatic Effects

Methods

Lawn plates of S. aureus, Pseudomonas aeruginosa, and E. coli of Trypticase Soy Agar (TSA) were prepared from suspensions of 18-hour cultures. Similar plates were made on Littman's media with Pityrosporum ovale, Candida albicans and Trichophyton mentagrophytes. One drop (1/40 ml) of various aluminum salts was then pipetted into the inoculated plates. The test agents were 1%, 10%, 20% and 30% concentrations (weight/volume) of aluminum chloride ($AlCl_3.6H_2O$), aluminum chlorhydroxide and aluminum acetate. A zone of no growth greater than 2 mm. indicated complete inhibition; clearing with sparse growth was regarded as partial inhibition. The plates were incubated for bacteria and yeasts for 24 hours at 35° C. T. mentagrophytes was allowed to grow for five days at room temperature.

Results

Aluminum chlorhydroxide at 1% inhibited completely all organisms except T. mentagrophytes.

Aluminum acetate completely inhibited all organisms at 10%.

Aluminum chloride was completely inhibitory to all organisms at 30%: at 20% there was partial inhibition of Pseudomonas and Pityrosporum with complete inhibition of the rest.

II. Bacteriocidal Effects

Methods

One ml. of a 1:100 dilution of a 24-hour culture was added to a 9 ml. of the above aluminum salts to give final concentrations ranging from 1:100 to 1:1,000,000. The test organisms were a cutaneous micrococcus (Baird-Parker type 3), a diphtheroid, C. minutissimum, Pseudomonas aeruginosa and Candida albicans. After 5, 10, 15 and

60 minutes, one ml. was removed and drop-plated on TSA agar and TSA agar with lecithin and polysorbate as detoxicants and incubated at 35° C for 48 hours.

Results

C. albicans and Pseudomonas were completely resistant to these concentrations of all aluminum salts.

Aluminum chlorhydroxide killed the micrococcus and diphtheroid out to the 1:1000 dilution.

Aluminum chloride was lethal to the micrococcus and diphtheroid only at the 1:100 concentration.

Aluminum acetate was lethal only to the micrococcus at the 1:100 concentration.

COMMENT

All three aluminum salts demonstrated broad spectrum antimicrobial activity but the potency varied greatly. The aluminum chlorhydroxide completely inhibited growth of all the test strains except T. mentagrophytes while comparable activity required 10% aluminum acetate and 30% aluminum chloride. Similarly, in the bacteriocidal assay, aluminum chlorhydroxide was effective at more dilute concentrations than the other salts. Blank et al. also found aluminum chlorhydroxide to be a potent bactericide. They stressed that 1.0% concentrations of aluminum salts were not very effective against gram negatives.

ASTRINGENT ACTIVITY

The term "astringency" cannot be precisely defined and probably encompasses multiple actions. It derives from clinical usage and measurements of this property are arbitrary. We used protein precipitation as a criterion for such activity.

Method

To two ml. of a 4% bovine albumin solution were added two mls. of the following: 30%, 20% and 10% $AlCl_3.6H_2O$, 30% aluminum acetate, 30% aluminum chlorhydroxide and 30% aluminum sulfate. Readings were made at five minutes, one hour and at 24 hours.

Results

30% $AlCl_3.6H_2O$ produced a thick precipitate within five minutes. With 20% there was turbidity at five minutes which became flocculant by 24 hours. The 10% solution had no effect.

30% solutions of the other salts produced no precipitation except for 30% aluminum chlorhydroxide which became turbid in 24 hours.

Comment

A major effect of astringents is to produce "dryness." This probably entails complexing with proteins, altering their ability to swell and hold water. Aluminum chloride was the only one of the salts tested that possessed protein-precipitating properties to a high degree. From this, one would expect it to be more drying. We found that callus immersed in 20% aluminum chloride took up only half as much water as the control.

IN VIVO ANTIBACTERIAL ACTIVITY

I. Bacteriostatic Effects

The bacteriostatic activity of 10, 20 and 30% aluminum chloride and 20 and 30% aluminum chlorhydroxide was compared on opposite forearms of ten volunteers. A volume of 0.1 ml of each concentration was applied to 5 cm. squares (25 sq. cm.).The application was immediately covered with identically-sized squares of impermeable film and sealed to the skin for 24 hours with plastic tape (Blenderm). A non-treated control was similarly occluded.

In another ten subjects, 10 and 20% aluminum acetate were compared to 1:20 and 1:40 dilutions of 5% Burows solution (aluminum subacetate); these are the recommended-use concentrations. The sites were sampled after 24 hours of occlusion.

Results

All the aluminum solutions except for the 1:20 and 1:40 dilutions of Burows solutions were bacteriostatic. 10% concentrations of aluminum chlorhydroxide and acetate usually sterilized the skin surface. It required 20 and 30% concentrations of aluminum chloride to approach this activity.

II. Bacteriocidal Effects

The forearms of ten volunteers were wrapped with impermeable plastic film from the elbow to the wrist for 48 hours. Immediately after removing the film, 0.1 ml of 10, 20 and 30% $AlCl_3.6H_2$) and 10 and 20% aluminum chlorhydroxide was applied to 5 cm. squares. These sites and a control area were then covered with plastic film and sealed to the skin for 48 hours as above. A similar procedure was followed in another ten subjects utilizing 10 and 20% aluminum acetate and 1:20 and 1:40 dilutions of 5% Burows solution.

Results

The chlorhydroxide was again the most potent salt and in fact sterilized the surface (TABLE XVII). Aluminum acetate and aluminum chloride had appreciablekilling effects. Aluminum acetate was more potent than the chloride at corresponding concentrations. Burows solution at both dilutions demonstrated no bacteriocidal activity.

Treatment of Interdigital Athlete's Foot with Aluminum Salts

Salts of aluminum were compared in the treatment of the symptomatic, wet form of "athlete's foot." These studies were conducted on prisoner volunteers in Philadelphia, during the summer months. Athlete's foot blooms at this time and there were many applicants. Subjects were selected who had equivalent bilateral involvement of moderate severity. Simple, dry dermatophytosis was excluded. Each material was liberally applied twice with cotton pad applicator. Mycologic and bacteriologic examinations were not done.

(1) In ten subjects 10% aluminum chloride was compared to 30% on opposite feet. In another ten, 20% was compared to 30%. 1o% had but slight effects. The 20% concentration was strongly drying in four and moderately drying in six accompanied by good clinical improvement. The 30% solution produced marked drying in all ten. All of these experienced a relief of pruritus and disappearance of malodor within two days. By a week, the signs of the disease had greatly abated.

(2) Ten subjects with bilateral, macerated athlete's foot comared 30% $AlCl_3.6H_2O$ and 30% aluminum chlorhydroxide. The interspaces treated with aluminum chlorhydroxide were not improved and the symptoms remained unchanged. Aluminum chloride again produced prompt symptomatic relief and stron improvement.

(3) Another ten subjects compared Burows solution as is (5% aluminum subacetate) to the recommended 1:20 dilution. After seven days

the 5% solution produced moderate drying and symptomatic improvement in five; the 1:20 dilution was ineffective.

(4) Ten subjects compared 30% $AlCl_3.6H_2O$to Castellani's paint. After one week, both sides showed equivalent improvement.

Comment

These were purely clinical trials. The results demonstrated that 30% aluminum chloride was superior to other familiar aluminum salts. The response was prompt both subjectively in decreasing pruritus and objectively in relief of maceration and elimination of malodor.

Effect of Aluminum Chloride on the microbiology of Athlete's Foot

Eleven patients with bilateral, wet, macerated, hyperkeratotic, interdigital lesions were studied. A 30% $AlCl_3 \cdot 6H_2O$ solution was applied with a cotton tip applicator twice daily for seven days. Semi-quantitative bacteriological data was obtained with a modification of the detergent scrub technique as follows: A sterile cotton-tipped applicator moistened with 0.1% Triton X-100 was vigorously rotated in the interspace and then placed in 1 ml. of 0.1% Triton X-100. The sample was processed as above and then the density of organisms per web space calculated.

Prior to treatment, the aerobic count was the order of tens of millions or organisms per interspace (TABLE XIX). A variety of aerobic diphtheroids, comprised the bulk of these. Cocci were abundant. Gram negatives were found in four, *S. aureus* in one and non-hemolytic Streptococci in two cases. Dermatophytes were cultured in five of the eleven. After seven days of applications, all eleven demonstrated at least a 75% clinical improvement subjectively and objectively. Bacteriologically, the total flora was suppressed to the level of hundreds of thousands of organisms per interspace; a reduction of 99%. The aerobic diphtheroids were drastically reduced and coagulase negative cocci became dominant in three who improved as much as the rest; the same dermatophyte was recovered after treatment. *S. aureus*, *Pseudomonas* and *Proteus* were also substantially reduced.

DISCUSSION

Two circumstances stimulated us to pursue non-conventional strategies for treating athlete's foot. Firstly, the newer agents, while potent fungicides, have not in our hands proved very efficacious in treating the symptomatic, odiferous, soggy macerated variety of this disease. Relief is slow and often incomplete. They are nicer than, but therapeutically inferior to, Castellani's paint. In what we take to be an example of British whimsy, Clayton and Comer found that Clofrimazole was as effective as half strength Whitfield's ointment! Others have obtained remarkable results in athlete's foot with imidezole derivatives, but we do not know their opinion regarding the venerated Whitfield's ointment. As we see it, the dry, scaly variety of athlete's foot is generally asymptomatic. It is only in this type that fungi can be regularly demonstrated by culture or microscopic examination. If therapeutic evaluations are carried out only on mycologically positive subjects, the majority of patients with symptomatic diseases are eliminated from the study. This is the reason, we think, that Hermann et al. were able to score such impressive results in tinea pedis with haloprogin and tolnaftate. To satisfy the requirements of the Food and Drug Administration, they could only include cases in which fungi could be demonstrated, that is, simple cases which are much easier to treat than those completed by bacterial overgrowth. Among combat troops in Vietnam, however, tolnaftate was used extensively but the results were hardly stunning. Knowledgeable observers considered that the value of such topical therapy in wet terrain was very doubtful. It is becoming clear that athlete's foot comprises a clinical spectrum ranging from asymptomatic infections with ringworm fungi to a severe, symptomatic disease in which fungi cannot be consistently demonstrated. Davis *et al*. recovered dermatophytes from 23% of normal interspaces. On the other hand, the failure to demonstrate fungi is anything near a majority of abnormal interspaces is the typical outcome in practically all surveys. Expert teams using modern methods rarely find fungi in more than a third of cases. Authorities such as Aijelio *et al*. diagnosed athlete's foot in 60% of 871 young men entering military service but demonstrated fungi in only 18%.

Similarly, in two studies conducted by Marples and Cuarsis only 11% and 6% of cases could be confirmed clinically from students of whom abnormal interspaces occurred in 60 and 70% respectively. Marples' interpretation is that dermatophytes do not initiate athlete's foot, instead hyperhidrosis causes maceration and scaling, creating a habitat which invites invasion by fungi. According to her, other disease becomes symptomatic when virulent bacteria enter the scene. *S. aureus* was recovered in 65% of symptomatic interdigital tinea pedis. Our experience is quite different. *S. aureus* can only occasionally be recovered among our severe cases, the dominant organism being resident diphtheroids. We find that fungi become very difficult to demonstrate when KOH positive feet are super-hydrated by occlusive dressings. In our studies, the dry, scaling variety is transformed into the wet, macerated type. Strauss and Kligman found previously that fungi tended to disappear when the feet were kept very wet. It has been repeatedly observed that upon transference from a temperate to a tropical climate, dermatophytes originally present in the toe web are no longer recoverable. Davis and his team have once again emphasized in military recruits, the poor correlation between clinical and cultural dermatophytosis.

In our view, interdigital athlete's foot usually begins with invasion of the horny layer by dermatophytes. With hot weather, sweating, exercise or tight shoes, enough moisture accumulates to stimulate an overgrowth of various bacteria. Gram negatives such as Pseudomonas and Proteus are responsible for the most serious cases while high numbers of normal residents, namely aerobic diphtheroids create the ordinary wet, macerated type. Alternation between the dry and wet forms in relations to moisture is commonplace experience. Acute flares are common in summer and can be experimentally induced by occlusion of fungus-infected feet.

Suppression of bacteria is an essential requirement in treating symptomatic athlete's foot. This can be accomplished in several ways. The simplest is exposure to air since moisture governs population density. However, wearing sandals and other tactics to enhance evaporation are often unfeasible. Antibacterial antibiotics might offer another approach. These would have to be active against gram negatives as well as gram positives. Indeed, drugs highly effective against the former would almost certainly induce a luxuriant growth of the latter. Agents which suppress gram positive organisms create favorable conditions for replacement by gram negatives. Pseudomonas infections are a serious consequence of such "one-way" ecologic pressures. The ideal, perhaps, would be a single agent with very broad spectrum coverage against dermatophytes, gram positives, gram negatives and Candida.

The imidozoles are broad spectrum compounds; the only difference being low activity against certain gram negatives. Perhaps when actively formulated in appropriate concentrations to suppress bacteria as well as fungi, this class of compounds will be more effective than the agents now available.

Our own candidate, aluminum chloride, combines broad spectrum antimicrobial activity with chemical drying, a two-pronged attack; we view drying as the decisive element. Aluminum chlorhydroxide is an effective anti-microbial agent but owing to feeble astringency, is far less effective.

It should be emphasized that rather high concentrations are required, 20 to 30%, to obtain a drying and antimicrobial effect. The marked superiority of aluminum chloride in drying up the soggy, macerated interspace is related to its ability to combine strongly with proteins. It has been shown that aluminum salts bind strong to delipized human keratinous materials, interestingly enough to callus more so than to stratum corneum. The horny layer in the interspace more closely resembles that of the palms and soles in comparison to the body surface. Of the salts we tested only $AlCl_3.6H_2O$ precipitated serum protein. This was not a pH effect; adjusting a concentrated solution of aluminum chlorhydroxide, originally pH4.5 to 2.2 (the Ph of 20% aluminum chloride) did not bring about flocculation. Neither aluminum sulfate nor the acetate possessed this property. Lansdown found that the topical application of aluminum nitrate and acetate caused no injury to the skin of rabbits and guinea pigs while aluminum chloride did. Likewise, we have been able to show (unpublished observations) that the nitrate and acetate are ineffectual as anti-perspirants. We are postulating a correlation between "astringency" (defined as protein precipitation) and various biologic effects, irritancy, drying and anti-perspirant ability. Aluminum chlorhydroxide, it must be observed, stands somewhere in between. It did produce some turbescence when mixed with protein. It is widely used as an anti-perspirant though inferior to aluminum chloride. Its acceptibility is based on lesser irritancy.

As regards irritation, we have encountered this only in three instances in an experience which now numbers scores of patients treated twice daily for a week or more. In each instance a fissure was present which enables the salt to directly contact living skin. Eroded or fissured skin are contraindications to the use of concentrated solutions of aluminum chloride.

Interdigital skin is more like the plantar surface with regard to the thickness of the horny layer, of the order of 250 microns compared to merely 15 for the stratum corneum of the abdomen. Despite greater permeability of plantar horn, its exceptional thickness is responsible for the high resistance of the skin of the soles to chemical irritants. Also, even with readily diffusing substances, the concentration reaching the visible epidermis is greatly reduced after traversing this thick horny layer. It turns out that aluminum salts, like most potent electrolytes penetrate skin poorly. Blank and Gould placed solutions of aluminum over excised human skin and could scarcely detect the material in the dermis. In human skin made antihidrotic by topical application of aluminum chloride, we identified aluminum histochemically in the ecerine sweat ducts but nowhere else except at the very surface. Keratin binding is another factor which would limit the diffusion of aluminum salts across the horny layer. This highly limited diffusion makes it unlikely that fungi deep within the horny layer could be eradicated by aluminum chloride. This agent is not very helpful in the simple, dry type of tinea pedis. Moreover, we sometimes found fungi persisting despite great clinical improvement. This again would emphasize that bacteria, not fungi, are dominant influences in soggy, symptomatic athlete's foot. Indeed, excessive moisture is in itself inimical to the fungus. When the fungus-bearing interspace of the dry type is artificially occluded, the dermatophyte diminishes or disappears and bacteria multiply to extremely high levels. This is always accompanied by an acute exacerbation and the development of white maceration. There is no indication that this resident bacteria which flourished in this wet phase invades the horny layer. It is fortunate that the bacteria are located mainly on the surface where they are accessible to aluminum salt.

We think, therefore, that aluminum chloride in high concentrations exerts its antibacterial effect in two ways: by direct killing and by making the interspace inhospitable through drying. Lack of moisture is the factor which on exposed areas such as the forearm keeps the bacterial population at very low levels. After immersion, plantar horny layer absorbs water to the extent of about 200% of its dry weight, meanwhile swelling considerably.

In the presence of 20% aluminum chloride, swelling does not occur appreciably and the weight increases by about 80%. These changes are reversible if the horny layer is immersed in plain water, suggesting that osmotic forces chiefly prevent swelling. Concentrated solutions of sodium chloride also prevent swelling but are of no use in moderating athlete's foot. Astringency is a vital factor in the efficaciousness of aluminum chloride.

We do not propose that concentrated aluminum chloride cures athlete"s foot. It shifts the disease back to the simple, dry type and provides immediate symptomatic relief. In this respect, it stands in conspicuous contrast to conventional antifungal remedies. Within a few days, there occurs marred domination of pruritus, odor, wetness and whiteness. The interspace, while not quite normal by a week, is certainly no longer a troublesome site. We find that once-daily application will insure control of symptoms after that. In hot, humid weather, stopping the applications will be followed by a return to the original condition in about seven to 15 days, if not sooner.

We doubt that any local treatment can permanently eradicate interdigital athlete's foot. No doubt, potent antifungal agents can virtually exterminate dermatophytes but the invariable presence of infection elsewhere, on the nails or on the soles, assures reinfection.

In shoe-wearing populations of temperate climates, interdigital tinea pedis is mainly a seasonal disease, flaring in the summer, sleeping in the winter. Aluminum chloride is a practical way to prevent or ameliorate hot weather exacerbations. It is also very helpful in cutting down the disease to a tolerable level whenever, again in shoe-wearers, bacteria-promoting, local wetness cannot be prevented.

We did not find aluminum chloride to be superior to Castellani's paint. Our only accomplishment is the substitution of a colorless solution.

TABLE XVII

ANTIMICROBIAL ACTIVITY OF ALUMINUM SALTS

Agent	S. aureus	Pseudomonas	E. coli	P. ovale	C. albicans	T. mentagrophyte
1% aluminum chloride	0	0	0	0	0	0
1% aluminum acetate	0	0	0	0	0	0
1% aluminum chlorhydroxide	++	++	++	++	++	0
10% aluminum chloride	0	0	0	0	0	0
10% aluminum acetate	++	++	++	++	++	++
10% aluminum chlorhydroxide	++	++	++	++	++	++
20% aluminum chloride	++	+	++	+	++	++
20% aluminum acetate	++	++	++	++	++	++
20% aluminum chlorhydroxide	++	++	++	++	++	++

0 = no inhibition
\+ = partial inhibition
++ = complete inhibition

TABLE XVIII

In vivo Inhibition of Resident Microflora by Aluminum Salts

	Static Activity	Cidal Activity
Control	1,070,000	3,526,000
10% aluminum chloride	510	153,421
20% aluminum chloride	50	10,500
30% aluminum chloride	10	3,175
20% aluminum chlorhydroxide	No growth	No growth
30% aluminum chlorhydroxide	No growth	No growth
10% aluminum acetate	10	568
20% aluminum acetate	No growth	195
1:40 Burow's	627,000	3,611,000
1:20 Burow's	450,000	2,640,000

* The values are the geometric mean of aerobic organisms per sq. cm. for ten subjects.

TABLE XIX

EFFECT OF 30% ALUMINUM CHLORIDE ON THE MICROBIOLOGY OF ATHLETE'S FOOT

(Density in Logs)

Dermato-phytes	Total Aerobes	Cocci	Diphtheroids	GNR	SA	Other	Dermato-phytes	Total Aerobes	Cocci	Diphtheroids	GNR	SA
0	7.6990	6.3010	7.6812	0	0		0	4.2833	4.2833	0	0	0
0	7.9031	6.5051	7.8808	0	0		0	6.2304	6.1761	5.3802	0	0
+	8.8116	0	8.8102	6.2553 (Proteus)	0		+	4.1818	4.1335	3.2041	0	0
+	8.4713	4.6021	8.4669	6.4314 (Proteus)	0		+	6.3010	6.2833	5.2041	0	0
0	8.2833	6.6990	8.2917	0	0		0	6.3945	6.3945	0	0	0
0	7.8739	6.6924	7.8222	0	0		0	6.3181	6.3181	0	0	0
+	8.2511	6.7482	8.4914	0	0	7.2041 (γ Strep)	+	4.6812	3.8572	4.6107	0	0
+	8.1461	6.6021	8.1303	0	0	6.000 (γ Strep)	0	4.9445	4.4346	4.7839	0	0
+	8.7419	7.6021	8.7084	5.3010 (Kleb)	0		0	6.8645	0	0	0	6.863
+	8.4722	8.0170	8.1335	0	0	7.6021	0	6.1206	6.8976	5.6990	0	6.681
0	7.4713	5.8451	7.3856	4.6128	0	6.6021	0	5.9031	5.7160	5.0792	0	5.079

GNR = Gram-negative rods
SA = *Staphylococcus aureus*
Kleb = Klebsiella

APPENDIX I

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1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Sustained Protection against Superficial Bacteria and Fungal Infection by Topical Treatment		5. TYPE OF REPORT & PERIOD COVERED Final Report 1 Sept. 1970 - 31 Aug. 1974
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Albert M. Kligman, M.D., Ph.D.		8. CONTRACT OR GRANT NUMBER(s) DADA 17-71-C-1009
9. PERFORMING ORGANIZATION NAME AND ADDRESS The Trustees of the University of Pennsylvania Philadelphia, Pennsylvania 19104		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS The Trustees of the University of Pennsylvania Philadelphia, Pennsylvania 19104		12. REPORT DATE 31 December 1974
		13. NUMBER OF PAGES 73
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

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bacteriostatic activity, bacteriocidal activity, paired-comparison, atopic dermatitis, psoriasis, Candida albicans, aluminum salts, athlete's foot, Staphylococcus aureus, Pseudomonas, Proteus, Corynebacterium minutissimum, dermatophytes

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This report summarizes investigations (1) delineating simple reproducible bioassays for measuring bacteriostatic and bacteriocidal activity of topical antimicrobial agents; (2) delineating pitfalls in using the paired-comparison type of analysis for topical antibacterials; (3) quantifying the bacteriology of two common dermatoses, atopic dermatitis and psoriasis, and the effect of antibiotic therapies in these disorders; (4) delineating experimental models for the evaluation of steroid-antibiotic combinations;

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No. 20 continued - (5) describing a technique for experimental *Candida albicans* infections and its usefulness in evaluating anti-candi[illegible] therapies; (6) describing the microbiology of the various species of interdig[illegible] eruptions of the feet and studies on various therapies for this conditio[illegible] [illegible]d (7) delineating *in vitro* and *in vivo* tests for the antimicrobial efficacy [illegible]nd stringent effects of various aluminum salts.

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